Original Article

Hepatoprotective Effect of Ethanolic Extract of *Capsicum Annuum L.* (Red Variety) on Paracetamol Induced Hepatotoxicity in Long Evans Rats

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ABSTRACT

Background: *Paracetamol induced hepatotoxicity is a critical problem. Plant products like Capsicum annuum, are known as an excellent source of flavonoids and phenolics, which are important antioxidant components that reduce the risk of such condition and ensures hepatoprotection.*

Objectives*: To evaluate the hepatoprotective effect of ethanolic extract of Capsicum annuum L. (red variety) and to compare its hepatoprotective activity with Silymarin.*

Methods: *This experimental study was performed on 35 rats which were taken for the study and divided in 7 groups, each group containing 7 rats. Hepatotoxicity was introduced by Paracetamol at a dose of 750 mg/kg every 72 hourly, and it was given to all except control group. Silymarin was given as standard. G-I rats served as control, G-II served as hepatotoxic control and given Paracetamol. G-III rats were given Silymarin at the dose of 50 mg/kg/day. G-IV and G-V rats were given extract of capsicum at a dose of 250 mg/kg and 500mg/kg daily. After completion of 21 days experiment, all the rats were sacrificed under chloroform anesthesia and blood and liver samples were collected. Biochemical parameters e.g., serum bilirubin, ALT and AST were estimated and histopathological examination of the liver of the rats was also done.*

Results: *Mean serum bilirubin, ALT and AST levels of paracetamol treated group increased significantly which were highest among all groups. All the parameters were improved with treatment of capsicum and the best result was observed with high dose of extract of capsicum which was similar to silymarin. Microscopic examination of liver tissues showed centrilobular necrosis, periportal fibrosis and loss of cellular architecture in G-II. Bridging fibrosis with improvement of necrosis were found in G-III and G-V.*

Conclusion*: Pretreated ethanolic extract of Capsicum annuum L. (red variety) has hepatoprotective activity, which is almost similar to Silymarin.*

Keywords: *Hepatotoxicity, capsicum, silymarin, paracetamol*

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INTRODUCTION

The liver is a major organ of human body which actively participates in different metabolic functions including metabolism of toxins, various synthetic products and medicines[1]. Hepatotoxicity may develop as a result of injury to the liver that is associated with impairment of liver function due to exposure to a drug or other substances and is the leading cause of withdrawl of a drug. Drug induced hepatotoxicity is one of the most common causative factors for both acute and chronic liver failure. Approximately 10% of acute liver failure and about

40-50% of all cases of liver injury are caused by different medicinal agents [2].

Paracetamol is a very popular and effective NSAIID, safe in normal therapeutic doses, used for the treatment of mild to moderate pain and for relieving fever. Near the end of 20th century, paracetamol took the place of aspirin which was a widely used over the counter (OTC) analgesic. It is now used for treating cancer pain as the first step of the World Health Organization (WHO) analgesic ladder[3]. Among the offending drugs, that causes hepatotoxicity, Paracetamol is most common which is responsible for predictable hepatic injury and the injury is manifested by elevation of serum amino transferases, serum billirubin with the features of hepatic necrosis in histopathology and reduced glutathione level[4].

The easy accessibility without the need of laborious pharmaceutical synthesis increased the recognition toward the plant derived herbal medicines. Drugs derived from plants, play a vital role in the management of liver diseases all over the world. A large number of plants and herbal extracts shows hepatoprotective effects. As a result, phytomedicines are now traditionally used in the liver disorders and are included in the complementary and alternative medicines[5].

Silymarin is obtained from a natural compound derived from the species *Silbyum marianum*, which is called Milk thistle. Silymarin has been used worldwide from long ago as an alternative medicine because of its beneficial role in the treatment of liver diseases as it possesses hepatoprotective and regenerative actions[6]. Capsicum is a member of the Solanaceae family and *Capsicum annuum* is mainly used commercially. Various types of capsicums are widely grown for the fruits that is eaten fresh and also processed to use in different preparations. They are excellent sources of antioxidants, folic acid, dietary fibres and energy and the red pepper possesses the highest content among all[7].Recently herbal drugs have gained importance and popularity because of their safety, efficacy and cost effectiveness. Therefore, importance has been given around the world to develop plant based hepatoprotective drugs effective against a wide range of liver diseases[8].

The study was structured to compare the hepatoprotective potential of the Ethanolic extract of *Capsicum annuum* L. red variety in two different doses with Silymarin in paracetamol induced hepatotoxicity in long Evans rats.

METHODS

This experimental study was carried out in the Department of Pharmacology, Sir Salimullah Medical College, Dhaka, Bangladesh, on long Evans rats with ethanolic extract of *Capsicum annuum* (capsicum) for 21 days.

The fresh fruits of *Capsicum annuum* red variety were purchased from local market and extract was prepared in the laboratory of Institute of Nutrition and Food Science, University of Dhaka. Tablet Paracetamol was obtained from Beximco Pharmaceuticals (BD) Ltd. and Capsule Silymarin was obtained from Square Pharmaceuticals (BD), Ltd.

A total number of 35 healthy adult Long Evans rats of both sexes, 8- 12 weeks of age, weighing approximately 100 to 125 grams, purchased from the animal house of the Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, were taken for this study. The rats were divided into 5 groups. Each group comprised of 7 rats and all the members were given standard lab diet. Group I was served as the normal control, Group II was the hepatotoxic control and treated by paracetamol 750mg/kg at every 72 hours interval. Group III was treated with Silymarin 50mg/kg daily with simultaneous administration of paracetamol 750mg/kg every 72 hourly. Group IV was treated with Ethanolic extract of *C. annuum* 250mg/kg daily simultaneously with paracetamol 750mg/ kg every 72 hourly and Group V was treated with Ethanolic extract of *C. annuum* 500 mg/kg daily simultaneously with paracetamol 750mg/ kg every 72 hourly. All the groups were treated for 21 days.

On 22nd day, animals were sacrificed under chloroform anesthesia and blood was collected by cardiac puncture. The blood samples were taken to the Department of Biochemistry of the same institution to analyze the biochemical parameters (e.g., serum

bilirubin, ALT, AST) by semi-autoanalyzer. After collection of blood, the livers were excised and about two third portions of livers were placed in 10% formalin containing separately labeled containers and taken to the Pathology department of the same institution for histological examination.

Data was collected in data collection form. The results were expressed as mean±SD (standard deviation). Statistical significance of differences between groups was determined by one way ANOVA test and Bonferroni test. The calculations were performed by using SPSS version 17.0 for windows. Results of intervention groups were compared with that of control group. The results were expressed as mean ± standard deviation. P values <0.5 were considered statistically significant. The study was approved by the Ethical Review Committee of Sir Salimullah Medical College, Dhaka, Bangladesh.

RESULTS

There was significant increase in serum bilirubin level in the paracetamol treated group (Group II). Groups treated with silymarin (Group III) and extracts of capsicum(Group IV and Group V) showed significantly lower serum bilirubin level compared to Paracetamol treated group and the level of high dose of capsicum treated group (Group V) is almost similar to silymarin. The induction of paracetamol significantly increased (P<0.001) the levels of serum enzymes ALT and AST. The levels of both enzymes were highest in Group II, the paracetamol treated group. The high dose of ethanolic extract of capsicum treated group (Group V) significantly (P<0.001) reduced the activities of the enzymes and the levels of the enzymes were nearly similar to the silymarin treated group (Group III).

Table I: *Serum bilirubin, ALT and AST levels in different groups of rats after completion of 21 days experiment (n=35)*

Groups	S. bilirubin (mg/dl)	ALT (IU/L)	AST (IU/L)
Group I (n=7)	0.86±0.14	36.29±3.15	37.71±2.43
Group II (n=7)	6.60±0.21	80.00±2.90	88.50±4.37
Group III(n=7)	2.12±0.19	40.86±3.02	51.86±3.02
Group IV(n=7)	3.87±0.15	59.00±2.45	71.71±3.90
Group V (n=7)	2.28±0.20	41.00±2.94	40.91±4.07
p-value	<0.001	<0.001	<0.001

Table II: *Grading of hepatic necrosis by Histopathological changes in the livers in different groups of experimental rats. (N= 7)*

Group	Treatment	Grade of hepatic necrosis					
		0	1	2	3	4	5
I(n=7)	Control	7					
II(n=7)	Paracetamol	0	2	3	2		
III(n=7)	Paracetamol+ Silymarin	3	4				
IV(n=7)	Paracetamol+Capsicum(250mg/kg)	2	3	2			
V(n=7)	Paracetamol+ Capsicum(500mg/kg)	4	3				

Grading of hepatic necrosis by Histopathological changes in the livers in different groups of experimental rats was done by the arbitrary scale of Walker[9]. According to the scale, Grade 0 indicates no evidence of necrosis, Grade 1 reveals scattered small foci of centrilobular necrosis, Grade 2, most centrilobular areas show foci of necrosis, Grade 3, more severe necrosis affecting almost all centrilobular areas, Grade 4, confluent centrilobular and midzonal necrosis and Grade 5 stands for massive necrosis with only a narrow zone of surviving hepatocytes. The hepatic architecture in the paracetamol treated group (Group II) showed massive necrosis involving centrilobular and mid zone with a narrow periportal zone of surviving hepatocytes. The high dose capsicum treated group showed repopulation of the hepatocytes with minimum or no evidence of necrosis which was indistinguishable from silymarin treated group.

DISCUSSION

The present study was carried out to evaluate the hepatoprotective effect of *Capsicum annuum L.* red variety. The hepatoprotective effects were tested on Long Evan Norwegian rats. Hepatotoxicity was induced by oral administration of paracetamol at the dose of 750mg/kg body weight in the study.

The fruit of capsicum is proved to possess antiulcer, anticancer, antioxidant, radical scavenging and hepatoprotective properties. The ripe fruits of red capsicum are good sources of antioxidants. Earlier studies by Priya and Anitha suggests the potent antioxidant properties of red capsicum[10]. So, the hepatoprotective effect rendered by Ethanolic extract of *Capsicum annuum* could be attributed to the presence of flavonoids and phenolic compounds. In the present study Ethanolic extract of capsicum was given to the group IV and group V of experimental animals at the dose of 250mg/kg and 500mg/kg body weight respectively.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic disease. The result of the present study showed significant increase of serum bilirubin level in paracetamol treated rats which indicates hepatocellular damage. Incorporation of capsicum in diet and treatment with silymarin significantly decreased the level of serum bilirubin indicates their role against paracetamol induced hyper bilirubinaemia. These findings are in well agreement with Priya & Anitha[10] and Erhirhie & Ekene[11].

Assessment of liver function was made by estimating the activities of serum ALT and AST activity, which are enzymes originally present in higher concentration in cytoplasm. These enzymes leak into the systemic circulation during necrotic cell damage. The elevation of these enzymes occurred in paracetamol treated group and the levels are significantly lower in capsicum and silymarin treated groups. This indicates stabilization of plasma membrane and repair of liver tissue. Similar decrease in ALT and AST levels were reported by Gyawali et al.[12] and Radhika et al.[13].

Histologic analysis of rat liver treated with paracetamol showed significant hepatotoxicity, characterized by inflammatory hepatic tissues, including the presence of moderate infiltration of neutrophils. There was extensive necrosis in most of the centrilobular areas, in all rats, after hepatotoxicity induced by paracetamol. These histologic alterations in structure of the livers were improved by treatment with silymarin and the extract of capsicum in high dose. These observations of the present study coincide with previous similar studies done by Yeasmin et al.[1] and Masoud[14].

Comparing the general features as well as the biochemical and histological findings in different groups of experimental animals, it is obvious that toxic effects in the liver produced by paracetamol can be prevented by pretreatment with ethanolic extract of *Capsicum annuum L.* red variety in high doses. Prevention of liver damage evidenced by decreased enzyme levels with higher dose of *Capsicum annuum L.* red variety which was almost same observed with Silymarin, which was used as a reference standard.

LIMITATIONS

Molecular components of the active ingredients of Capsicum annum were not identified. Only Ethanolic extract of *Capsicum annum* was used. Different types of extract would reveal more information; but due to time and resource constrain more elaborate study could not be done.

CONCLUSION

The result of the present study provides a basis for the use of *Capsicum annuum* in the development of new herbal medicine suggesting its use as a potent hepatoprotective agent. Future works could be better directed towards obtaining the specific ingredient and the specific mechanism.

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